



Review

DNA barcoding to promote social awareness and identity of neglected, underutilized plant species having valuable nutritional properties

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ABSTRACT

It is estimated that about 7000 plant species and a large number of cultivars and varieties have been cultivated for consumption in human history. However, < 0.5% of these currently provide the majority of human food energy needs worldwide (e.g., rice, wheat, maize, and potato). Global issues such as climate change, diffusion of pests, and resistance to agrochemical treatments are posing great concern about the sustainable cultivation of these major staples, especially in equatorial and tropical countries, such as Sub Saharan Africa. In addition, most of these regions contain malnutrition and micronutrient deficiencies, and the sum of such problems create serious implications at social, political, and economic levels. A possible solution relies on the exploitation of plant biodiversity and particularly on the so-called NUS (Neglected and Underutilized Species). These plants are traditionally grown in their centres of origin and continue to be maintained by sociocultural preferences, however they remain inadequately documented and neglected by formal research and conservation programs. Although they are important in terms of micronutrients and the ability to grow in harsh conditions, these species are falling into disuse due to agronomic, genetic, economic, and cultural reasons.

To promote and spread their cultivation at the global scale, along with knowledge on their suitability for human nutrition, reliable identification systems are necessary to guarantee adequate authenticity along the entire supply chain and distribution network. A precise identification of the different species and their varieties is fundamental both to retrieve information on their origin and authenticate the raw materials (i.e., seeds, leaves and fruit) and related processed products that can be distributed at the local or global scale. DNA-based techniques can help achieve this mission. In particular, the DNA barcoding approach has gained a role of primary importance due to its universality and versatility. Here, we discuss the advantages in using DNA barcoding for the identification of some of the most representative NUS species, as well as their traceability and conservation of cultural practices around them.

1. Introduction

1.1. From plant domestication to the opportunity and critical issues of the Green Revolution

The domestication of wild plants has been a pivotal step in human history that determined the shift from hunter-gatherers to agricultural societies and stimulated the rise of cities and modern civilization. Humans still rely on the same crops that were domesticated around 10,000 years ago in different regions of the world such as Central America, New Guinea, and the Fertile Crescent (Purugganan & Fuller, 2009). From these areas, most of the cultivated plants spread worldwide due to the progressive globalization process (Zeder, 2008).

Despite the relevance of agriculture, two-thirds of global plant-derived food is provided by only three major crops – maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*). Our dependence on such crops limits our capability to deal with challenges posed by the adverse effects of climate change and the consequences of dietary imbalance (Cheng, Mayes, Dalle, Demissew, & Massawe, 2017).

From the 1960s through the 1980s, the so called Green Revolution focused on improving agronomic and nutritional features of the major staple crops (e.g., maize, rice and wheat) diffused during primary and secondary domestication processes. The principal goals were the enhancement of plant productivity and increasing the content of macronutrients, such as carbohydrates and proteins (Evenson & Gollin, 2003). Most of the plant varieties selected during the Green Revolution were

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also methodically bred to deal with emerging environmental and biotic stresses, and they were expected to produce yields several times higher than local cultivars. Unfortunately, the problems of modern agriculture remained largely unresolved. In particular, the diversity of crops and their varieties is decreasing, and the general trend of farming intensification led to the concentration of pests, thus causing a general loss of productivity for the target species of the Green Revolution (Pingali, 2012). Given these conditions, farmers are more and more asked to select new crop varieties providing enhanced yields and resistance to pathogens and environmental stresses, such as desertification. A putative solution to these problems could be represented by the introduction of Genetically Modified Organisms GMO. However, this approach could not be economically sustainable in those areas characterized by high levels of poverty (Bazuin, Azadi, & Witlox, 2011). A more effective approach, in terms of both the economic and environmental sustainability, is represented by the adoption of ecological intensification practices (Kovács-Hostyánszki et al., 2017). In this framework it could be possible to enhance crop yields by supporting for example pollinators' density and diversity and to tackle pests and pathogens occurrences by sustaining the communities of their natural enemies (Garibaldi et al., 2016). Furthermore, the adoption of plant species and varieties well-adapted to water scarcity could act as a reliable strategy in conditions of desertification (Mabhaudhi, Chimonyo, Chibarabada, & Modi, 2017).

The first category of problems affecting modern agriculture are those related to climate change (Zilberman, Lipper, McCarthy, & Gordon, 2018). With the average global temperatures predicted to increase above 3.0 °C by midcentury, agricultural productivity is expected to significantly decline in many countries (Abraham et al., 2014) as has already been demonstrated for many important staples, notably maize, wheat, and rice, due to their sensitivity to water shortages and heat stress (Khoury et al., 2014; Wheeler & Von Braun, 2013). Additionally, the lack of diversity within gene pools in modern crops reduces the possibility of producing new resistant genotypes and leaves the current agricultural systems vulnerable to the global issues mentioned above (Fuller et al., 2018; Zilberman et al., 2018). A second issue of global concern in agriculture is related to the nutritional properties of the cultivated species and their effect on human health. Major crops for example are unbalanced in terms of carbohydrates vs. micronutrients (Burchi, Fanzo, & Frison, 2011). An opportunity comes from those regions not interested by the Green Revolution, or at least where it did not impact consistently. For example, Sub-Saharan Africa experienced a lag in the benefits provided by the modern crop varieties of Green Revolution due to the political and pedoclimatic conditions (Ejeta, 2010). Therefore, there are many autochthonous edible plants that could be seen as unexplored items in the context of agrobiodiversity and that could contribute to improve agricultural issues worldwide. For example, some legumes such as the pigeon pea *Cajanus cajan* are rich in proteins, carbohydrates and micronutrients, and due to their limited spread are more resistant to environmental stresses such as pests, scarcity of water and fertilization (Odeny, 2007; Saxena, Kumar, & Sultana, 2010). Similarly, some green leafy vegetables such as *Amaranthus* spp. and *Solanum* spp. (e.g. *S. aethiopicum*, *S. macrocarpon*, *S. melongena* and *S. scabrum*) are well diffused in Sub Saharan countries and are more resistant to climatic stress than the well-known and largely cultivated common spinach (*Spinacia oleracea*). These species are also richer in micronutrients such as vitamins and minerals (Chivenge, Mabhaudhi, Modi, & Mafongoya, 2015). Although their agronomical and nutritional potential, the main problem of such local African species is related to their current use and diffusion which are often scarce and limited to local markets or even to achieve subsistence at the family level. For these reasons, such plants have been generally referred to as Neglected Underutilized Species (NUS). The most common definition of NUS refers to those crops excluded from the scientific research, from the improvements of the Green Revolution and from the main distribution chain (Hammer, Heller, & Engels, 2001; Naveena, Mouzam, &

Bellundagi, 2016; Padulosi, Thompson, & Rudebjer, 2013). Nowadays, the term NUS could also be extended to every alternative use of a crop or their portions. This latter point refers for example to the cowpea (i.e. *Vigna unguiculata*) which seeds are usually the target of consumption, but in Southern African countries its leaves are also eaten cooked or dried (Ahenkora, Dapaah, & Agyemang, 1998).

The main reasons that limit the expansion of these crops concern agronomic, genetic and economic issues (Njeru, 2016; Padulosi et al., 2013). Regarding the first two aspects, the NUS can be considered as proto-domestic species, since they have not been selected during the centuries on the basis of their higher productivity or seeds dimension, as in the case of major staple crops (Snir et al., 2015). Moreover, NUS often lack a complete food supply chain supporting their commercialization (Will, 2008). Multidisciplinary studies and programs linking informatics, genetic analysis, and traditional knowledge are increasing the awareness and the access to NUS (Dansi et al., 2012; Padulosi, Bergamini, & Lawrence, 2012). In some cases, innovative farming strategies of NUS and dedicated breeding programs increase the diffusion and the market value of these plants (Neugart, Baldermann, Ngwene, Wesonga, & Schreiner, 2017); however, in most cases NUS and knowledge around them are scarce, partial or progressively disappearing (Mellisse, Descheemaeker, Giller, Abebe, & van de Ven, 2018).

To preserve NUS biodiversity and enhance their utilization, it is necessary to stimulate the dialogue among farmers, scientists and final consumers through a participatory approach aimed at improving smallholder agroecosystems, diets, livelihoods, and global food security (Afari-Sefa et al., 2016). Enhancing the nutritional and agronomical positive features of NUS and implementing their use will hopefully lead to the creation of a supply chain that supports the commercialization of such species (Mabhaudhi et al., 2017). This scenario requires a precise knowledge of the involved species and of their traditional uses. However, in many cases, each crop identified by a common name (e.g., amaranth or nightshade) could encompass an astounding list of varieties and different species or hybrids. It is therefore essential to rely on efficient analytical systems to provide a unique identification of NUS plants and related food items.

1.2. Discovering the NUS

The term NUS refers to a large panel of species including legumes, grains, fruit, and vegetables adapted to a wide panel of environmental conditions mainly related to drought and soils poor in organic matter and micronutrients. The higher resilience of NUS to harsher conditions than the major cereal crops, make them suitable to grow in low-input farming systems in marginal environments and to face the environmental stress conditions posed by climatic change issues. Many African NUS such as *Cleome gynandra* and *Amaranthus* spp. are C4-photosynthetic species (Marshall et al., 2007; Tsutsumi, Tohya, Nakashima, & Ueno, 2017) therefore they can save more water than the common C3-based crops (Griffiths, Weller, Toy, & Dennis, 2013). For these reasons, plants like the quinoa (*Chenopodium quinoa*), once considered NUS, have recently received research attention and dedicated breeding programs to enhance their quality and productivity features (Chivenge et al., 2015), since they are more resistant than other staple crops to water scarcity (Jacobsen, 2003). Furthermore, NUS are suitable for intercropping with staple crops, enhancing crop productivity and nutrient-use efficiency and providing at the same time the basis for a better balanced diet (Ebert, 2014).

Another important characteristic of such plants refers to their higher nutritional properties in terms of macro and micronutrients content with respect to those of common staple crops (Nyadanu & Lowor, 2015; Padulosi et al., 2012). Micronutrients deficiency is a globally shared problem as in the case of iron, vitamin A, and zinc. Iron deficiency in developed countries is treated by introducing more meat and some iron-rich vegetables (e.g., *S. oleracea*) into the diet. Interestingly, some NUS,

Table 1

- Examples of NUS providing high contents of micronutrients and secondary compounds and comparison with values of common and widespread staple crops. To provide an immediate comparison, only the maximum concentration of each micronutrient/secondary compound is reported. (FW = fresh weight, DW = dry weight, PE = protein extract).

Species	Origin	Common name	Edible portion	Most abundant micronutrients per 100 g	Principal metabolites per 100 g	Comparison with representative staple crops	References
<i>Amaranthus sp</i>	America	Amaranth	Leaves boiled	Ascorbic acid 1.26 mg; Ca 425 mg; Mg 224 mg FW	Betalains 7.73 mg FW	Ascorbic acid > 2 times <i>Citrus x sinensis</i> , Ca 2 ~ times <i>Brassica oleracea</i> , Mg > 2.5 times <i>Spinacia oleracea</i> , betalains higher than quinoa (0.15–6.1 mg/100 g)	Kruger, Sayed, Langenhoven, & Holing, 1998; van Wick & Gericke, 2000; Steyn, Olivier, Winter, Burger, & Nesamvuni, 2001; Maughan et al., 2012; Biswas, Dey, & Sen, 2013; Abderrahim et al., 2015
<i>Cleome gynandra</i>	sub-Saharan Africa, Asia	Spider plant	All young plant's part cooked as vegetables (the leaves may also be dried after cooking)	β -carotene 3 g; Fe 2.9 mg; Folate 346 μ g FW	Glucosinolates 294 mg FW	β -carotene > 100 times <i>Ipomoea batatas</i> , Foliates ~ 2 times <i>Lens culinaris</i> , glucosinolates > 10 times <i>Brassica rapa</i> (20 mg/100 g)	Kruger et al., 1998; van Wick & Gericke, 2000; Chen, Zhu, Gerendás, & Zimmermann, 2008; Stoessel, Juraskie, Pfister, & Helliweg, 2012; Onyango, Kunyanga, Onttia, Naria, & Kimenjitu, 2013; Omondi et al., 2017; Wu, Solberg, Yndgaard, & Chou, 2018
<i>Corchorus olitorius</i>	Africa	Jute mallow	Leaves (cooked) eaten with porridge	Ascorbic acid 105 mg, β -carotene 5 g, Ca 265 mg, Fe 20–54 mg, Foliates 100 μ g, Zn 5 mg FW	Polyphenols 0.25 g FW	Ascorbic acid 1.39 times, Ca, Fe > 3 times <i>S. oleracea</i> ; polyphenols > 1.5 S. <i>oleracea</i> (0.15 g/100 g)	van Wick & Gericke, 2000; Roy et al., 2006; Hanif, Iqbal, Iqbal, Hanif, & Rasheed, 2006; Stoessel et al., 2012; Ko et al., 2014; Nyadanu & Lowor, 2015; Giro & Ferrante, 2016
<i>Galinsoga parviflora</i>	South America	Gallant Soldier	Leaves	Ca 162 mg, Mg 681 mg, Mn 44 mg FW	Polyphenols 3.5 g DW	β -carotene > 200 times <i>I. batatas</i> , Mg 10 times <i>S. oleracea</i> ; polyphenols higher than <i>S. oleracea</i>	Udosen, Udok, & Unuigbo, 1999; van Wick & Gericke, 2000; Odhav, Beekrum, Akula, & Bajinath, 2007; Ranilla, Kwon, Apostolidis, & Shetty, 2010; Ko et al., 2014
<i>Justicia flava</i>	Africa	Yellow justicia	Leaves and preboiled young seeds	Ca 2073 mg, Fe 16 mg, Zn 11 mg DW	Lignans (amount not available)	Incredible source of Ca: > 20 times higher than in <i>S. oleracea</i>	Olaniyi & Powell, 1980; van Wick & Gericke, 2000; Hanif et al., 2006; Odhav et al., 2007
<i>Labiab purpureus</i>	India	Labiab	Leaves and preboiled young seeds	α -tocopherol 2.77 mg, Ca 58.5 mg, Fe 6.78 mg, Zn 2.66 mg, FW	Aminoacids 18.25 g DW	Total tocopherol content higher than cereals, quinoa and common bean, Zn 2 times <i>Phaseolus vulgaris</i>	van Wick & Gericke, 2000; Rychlik, Englert, Kapfer, & Kirchhoff, 2007; Habib, Al Meqbali, Kamal, Souka, & Ibrahim, 2014
<i>Lasianthera africana</i>	Africa	-	Leaves	Ca 800 mg, Fe 77 mg, Zn 36 mg FW	Polyphenols 1.821 g DW	Ca ~ 10 times, Fe > 3 times <i>S. oleracea</i>	Udosen et al., 1999; van Wick & Gericke, 2000; Hanif et al., 2006; Atiko, Onocha, & Oyedemi, 2016
<i>Launaea cornuta</i>	Africa	Bitter lettuce	Leaves	Ascorbic acid 15.9 mg, Ca 265 mg, Fe 2.7 mg FW	Many antimalarial compounds (e.g. polyphenols, amounts not available)	Ca 3 times <i>S. oleracea</i>	van Wick & Gericke, 2000; Lyimo, Temu, & Mugula, 2003; Hanif et al., 2006
<i>Senna occidentalis</i>	South America	Coffee senna	Seeds and leaves	Ca 513 mg, Fe 3 mg, Zn 2 mg FW	Aminoacids 62.13 g PE	Aminoacids and Zn 2 times <i>P. vulgaris</i>	van Wick & Gericke, 2000; Henderson, 2001; Odhav et al., 2007; Rychlik et al., 2007; Oshoke & Akinyemi, 2015
<i>Solanum aethiopicum, nigrum, scabrum, macrocarpon, dulcamara</i>	South America	Nightshade	Leaves, fruit	Ascorbic acid 75 μ g, β -carotene 5.8 μ g, Ca 194 mg, Fe 3 mg, Folate 70 μ g FW	Polyphenols 42.1 g DW	Ca > 2.5 times; polyphenols ~ 300 times <i>S. oleracea</i> (0.15 mg/100 g)	van Wick & Gericke, 2000; Hanif et al., 2006; Ko et al., 2014; Ronoh et al., 2018
<i>Tylosima esculentum</i>	South Africa	Marama bean	Seeds	α -tocopherol 2.88–3.77 mg, Ca 93.7–217.6 mg FW	Phenolic acids and flavonoids 406.6 mg FW	Superior in composition to soya beans	van Wick & Gericke, 2000; Holse, Husted, & Hansen, 2010; Chingwaru, Vidmar, Kapewangolo, Mazimba, & Jackson, 2015
<i>Vernonia amygdalina</i>	Africa	Sweet bitterleaf	Leaves	Ascorbic acid 46.64 mg, β -carotene 9.05 mg, Ca 1264.1 mg, Fe 32.2 mg DW	Flavonoids 11.5 g DW	Ca, Fe and flavonoids higher than <i>S. oleracea</i>	van Wick & Gericke, 2000; Bergquist, Gertsson, Knuthsen, & Olsson, 2005; Hanif et al., 2006; Usunobun & Okolie, 2015
<i>Vigna subterranea</i>	Africa	Bambara groundnut	Seeds	Ca 220 mg, Fe 7 mg, Zn 8 mg FW	Aminoacids 68.6–76.6 g PE	Aminoacids > 2 times, Zn 8 times <i>P. vulgaris</i>	van Wick & Gericke, 2000; Massawe, Dickinson, Roberts, & Azam-Ali, 2002; Olatokun, Olorunfemi, & Akintayo, 2006; Rychlik et al., 2007

(continued on next page)

Table 1 (continued)

Species	Origin	Common name	Edible portion	Most abundant micronutrients per 100 g	Principal metabolites per 100 g	Comparison with representative staple crops	References
<i>Vigna unguiculata</i>	Africa	Cowpea	Seeds, leaves	Ca 66.4 mg, Fe 6.7 mg, Folate 149–152 µg, Mg 54.6 mg, Zn 6 mg FW	Aminoacids 63.9 g PE	Aminoacids > 2 times, Zn 6 times <i>P. vulgaris</i> (16), Folate 1.5–15 times <i>Pisum sativum</i> (10–87 µg/100 g)	Olaleke et al., 2006; Rychlik et al., 2007

such as *Corchorus olitorius* and *Vernonia amygdalina* are richer in iron than common staple crops such as spinach (Table 1). Concerning vitamin A, the global deficiency in children of pre-school age is about 25% (FAO, 2017) and the main source of this vitamin in staple crops is represented by potatoes and carrots. However, some dark leafy green vegetables from the Sub Saharan areas such as *Corchorus olitorius* and *Cleome gynandra* (Table 1) are richer in this and other vitamins rather than globally diffused staples.

Other important compounds of nutraceutical interest are aminoacids, which are highly represented in many NUS legumes, as *Vigna subterranea*, *Vigna unguiculata* and are two times higher than the common bean *Phaseolus vulgaris* (Table 1).

Concerning plant secondary metabolites, given their importance in the modern diet for health and wellness, it is important to underline that a diet rich in traditional leafy vegetables such as spider plant, jute mallow and amaranths could promote the uptake of many healthy-promoting compounds, such as glucosinolates, polyphenols and betalains. Glucosinolates are secondary compounds typical of the Brassicales showing great anti-inflammatory and antioxidant activities. They are 10 times more concentrated in *Cleome gynandra* than in the staple crop *Brassica oleracea* (Table 1). Polyphenols are compounds widely spread in the plant kingdom but they are of high-added value for their nutraceutical and pharmacological interest. Leaves of traditional NUS such as *Corchorus olitorius* or *Vernonia hymenolepis* are richer in polyphenols than the common staple *Spinacia oleracea* (Table 1). Betalains are secondary compounds of interest for their anti-inflammatory activities and are typical of the Caryophyllales. In amaranths, they are more concentrated than the common staple *Chenopodium quinoa* (Table 1).

NUS represent an important opportunity not only for their nutritional properties, but also for local socio-economic development. Many local NUS have the potential for being transformed in novel food products ready for distribution and are able to generate economic incomes for communities of smallholder farmers (Grùère, Giuliani, & Smale, 2008; Kahane et al., 2013). The introduction of NUS also constitutes a way to reduce the risk of over-reliance on very limited numbers of major crops and a way to increase sustainability of agriculture through a reduction in inputs, such as fossil fuel-derived and nitrogen fertilizers, given the high impact of the carbon footprint of agriculture on climate change (Ebert, 2014; Will, 2008).

International bodies, such as the World Vegetable Center (<https://avrdc.org/>) have the mission of finding and safeguarding many NUS and the cultural aspects related to their cultivation and use. For example, this center is working actively to improve *Amaranthus* ssp. and *Vigna subterranea*, and also distribute seeds to local farmers. Although well distributed locally, the same plants could be considered NUS at the global level and their poor marketability and limited access make them largely underutilized in both social and economic terms (Padulosi & Hoeschle-Zeledon, 2004). The worldwide agricultural sector needs to recognize the importance of NUS to enhance food security issues (Mayes et al., 2011) and to guarantee the transmission of traditional knowledge about their uses to the future generations.

The first step to valorize the NUS at the global level and enhance their diffusion is the development of a suitable species identification system, as well as the identification of their nutritional features. These plants are generally proto-domestic or spontaneous in local contexts and it happens that the same species could be known under different common names. This is the case for example of spider plant and African cabbage which refers to the same NUS, *Cleome gynandra* (Omondi et al., 2017). Conversely, different species can be grouped under the same common name such as *Amaranthus viridis* and *A. dubius*, that are usually referred to as Amaranth.

Among the available tools for species identification and traceability, DNA barcoding is the most universal, cheap, and inclined to automatization and rapid application as supported by a huge number of studies (see for example Galimberti et al., 2014; Hollingsworth,

Graham, & Little, 2011; Mishra et al., 2016). Therefore, in this review we intend to remark the advantages of universal molecular tools (such as the DNA barcodes), in promoting some species from a neglected and underutilized situation to a relevant role in modern agriculture and to authenticate their seeds and related food items. This could represent one of the main concrete actions to support Sub Saharan NUS as an element of global importance for a more sustainable and healthy diet.

2. DNA barcoding to address NUS authentication

In the last decades, DNA-based methods have been used to certify and trace several cultivated plants, such as rice, corn, barley, rye, and grape. Since 2003, a universal method known as DNA barcoding has been progressively diffusing to authenticate wild and cultivated species (Casiraghi, Labra, Ferri, Galimberti, & De Mattia, 2010; Galimberti et al., 2013). This is a simple and cost-effective (< 1\$ per sample, Meier, Wong, Srivathsan, & Foo, 2016) tool based on the characterization of universal DNA regions able to unequivocally identify organisms at the species level. As a general principle, ideal DNA barcode regions should have a high interspecific and low intraspecific variability. However, in some cases (e.g., De Mattia et al., 2011; Enan & Ahmed, 2014) this approach also allowed to characterize cultivated species at deeper taxonomic levels such as subspecies and cultivar.

In the case of metazoans, the ideal barcode region is a 658 bp portion of the mitochondrial COI (Casiraghi et al., 2010), while in plants the choice of the best barcode region/s was motivated by the guidelines of the International DNA Barcoding Plant Working Group (IDBPWG), that encouraged the use of the plastidial *rbcl* and *matK* regions (Hollingsworth et al., 2009). Furthermore, the plastidial intergenic spacer *trnH-psbA* and the nuclear ITS region were progressively adopted due to their higher variability (that permits the differentiation among congeners) and the already documented success in identifying herbal and traditional medicine products (De Mattia et al., 2011; Galimberti et al., 2014; Mezzasalma et al., 2017).

Although the molecular approach at the basis of DNA barcoding is not new to science, the strength of this method relies on the availability of reference sequences, belonging to morphologically recognized voucher specimens, that are archived by international platforms such as the BOLD (Barcode of life database) system. Reference barcode sequences are indeed the key stone for a correct identification and the starting point for the discovery of new entities (Casiraghi et al., 2010). BOLD is a public repository supporting the collection of DNA barcodes, with the aim of creating a reference library for all living species (Ratnasingham & Hebert, 2007). BOLD is used to assign a given DNA barcode to both a vouchered specimen, validated by expert taxonomists, and other DNA barcode sequences belonging to the same or different taxa. The lack of reference sequences is the main limit of the method. While some group of organisms are well represented in such databases, a lot of work is still necessary to provide a reliable source of reference DNA barcodes for groups which have been poorly investigated. Given the need for reference DNA barcodes to improve the reliability of the identification and the lower public resonance concerning NUS, we advocate for the creation of a dedicated NUS dataset that could serve as both a reference for identification and a repository for the conservation of these species. However, nowadays, many reference sequences for the barcode regions ITS2, *matK* and *rbcl*, necessary for NUS identification, are available in public international databases such as GenBank-NCBI (<https://www.ncbi.nlm.nih.gov/nucleotide>), as reported in Table 2. Looking at the species listed in this table, it is possible to find some examples of possible application of DNA barcoding in the case of NUS. In many cases, given subtle morphological similarities, it could be difficult to discriminate among species belonging to the same genus such as in *Amaranthus* (Achigan-Dako, Sogbohossou, & Maundu, 2014), or even among species belonging to different genera when the edible portions of the plant have been processed. This is case for example of *Launaea cornuta*, which can be easily replaced by *Sonchus oleraceus* or *Launaea*

Table 2
DNA barcoding characterization of NUS. For each species or genus, the distribution, the availability of published DNA barcoding studies and of reference barcode sequences in the international GenBank is reported.

NUS	Distribution	Topic of investigation	Reference DNA barcodes	References
<i>Amaranthus</i> spp.	worldwide	Species authentication	a) ITS2 - b) <i>rbcl</i> - <i>matK</i>	a) Kuzmina et al., 2017; b) Burgess et al., 2011
<i>Cleome</i> sp.	worldwide	Biogeography	a) ITS2 - b) <i>rbcl</i>	a) Feodorova, Voznesenskaya, Edwards, & Roalson, 2010; b) Cardinal-McTeague, Sytsma, & Hall, 2016
<i>Corchorus olitorius</i>	worldwide - tropical	Genetic evolution - Species authentication	a) ITS2 - b) <i>rbcl</i> - <i>matK</i>	a) Benor, Fuchs, & Blattner, 2011; b) Xu, Li, & Jin, 2018
<i>Galinsoga parviflora</i>	worldwide	Phylogeny - Species authentication	a) ITS2 - <i>matK</i> - b) <i>rbcl</i>	a) Blösch et al., 2009; b) Xu et al., 2018
<i>Justicia flava</i>	Africa	Taxonomy	ITS2	Kiel, Daniel, Darbyshire, & McDade, 2017
<i>Lablab purpureus</i>	worldwide - tropical	Species authentication - Phylogeny	a) ITS2 - <i>rbcl</i> - b) <i>matK</i>	a) Kuzmina et al., 2017; b) Stefanović, Pfeil, Palmer, & Doyle, 2009
<i>Lasianthera africana</i>	Africa	Species authentication	<i>matK</i> - <i>rbcl</i>	Parmentier et al., 2013
<i>Launaea cornuta</i>	Africa	Phylogeny	ITS2 - <i>matK</i>	Kim, Chunghee, & Mejías, 2007
<i>Senna occidentalis</i>	worldwide - tropical	Species authentication - Adulteration product	a) ITS2 - b) <i>matK</i> - <i>rbcl</i>	a) Mao et al., 2017; b) Seethapathy et al., 2015
<i>Solanum aethiopicum</i> , <i>nigrum</i> , <i>scabrum</i> , <i>macrocarpum</i> , <i>dulcamara</i>	worldwide	Phylogeny - DNA barcoding	a) ITS2 b) <i>rbcl</i> - <i>matK</i>	a) Siikinen, Barboza, & Knapp, 2015 b) Group et al., 2011
<i>Tylosene esculentum</i>	Africa	Plant physiology	<i>rbcl</i>	Mitchell, Keys, Madgwick, Parry, & Lawlor, 2005
<i>Vernonia amygdalina</i>	Africa	n.a	n.a	n.a
<i>Vigna subterranea</i>	Africa	Genetic diversity - Phylogeny	a) ITS2 - <i>rbcl</i> - b) <i>matK</i>	a) Takahashi et al., 2016; b) Wojciechowski, Lavin, & Sanderson, 2004
<i>Vigna unguiculata</i>	worldwide - tropical	Genetic diversity - Phylogeny	a) ITS2 - b) <i>matK</i> - c) <i>rbcl</i>	a) De Luca, Cennamo, Del Guacchio, Di Novella, & Caputo, 2018; b) Wojciechowski et al., 2004; c) Stefanović et al., 2009

taraxacifolia (Denton, 2004). A similar risk regards the genus *Senna* for which many different species could be adopted to prepare traditional health remedies. Seethapathy et al. (2015), reported the possibility and the need to distinguish *Senna* species through the DNA barcoding approach, since market analysis revealed frequent adulteration events (Seethapathy et al., 2015).

Preventing adulteration is of particular interest especially for processed NUS products such as dried leaves or flour. This is the case of *Vigna unguiculata* and *Cleome gynandra*, which are traditionally dried for conservation, or the *Vigna subterranea* which is often stored as flour. In small and medium farms of developing countries, adulteration could also derive from accidental substitution operated by seed distributors (even including governmental bodies, NGOs, small companies) and not from intentional frauds due to economic advantage. As stated by Barriga and Fiala (2018), recent efforts by governments and donors to address the problem of seed quality has been tending to focus on certification and labeling and to reduce the possibility of adulteration by downstream sellers (see also Louwaars & De Boef, 2012). Such products are morphologically unidentifiable and only a DNA-based approach could better clarify the identity of the original raw material. A similar situation recently occurred in the field of traditional (or neglected) medicinal plants, where remarkable progresses have been made through the use of DNA barcoding to promote their exploitation and the globalization of their market (see for example the reviews by Techen, Parveen, Pan, & Khan, 2014; de Boer, Ichim, & Newmaster, 2015; Raclariu, Heinrich, Ichim, & de Boer, 2018).

To date, most NUS lack of reference DNA barcoding accessions such as *Vernonia amygdalina*, *Justicia flava* and *Tylosema esculentum* and probably for many other NUS not mentioned in this study. This underlines the need to encourage the production and submission of reference accessions of NUS to enhance the adoption of the DNA barcoding as a standard tool to support NUS traceability along the entire supply-chain. An efficient molecular identification approach would enhance not only food safety issues but also it will support the regulation of the exploitation local genetic resources. In this context, the Nagoya protocol, adopted on 2010 by the contracting states to the United Nations Convention on Biological Diversity (CBD), represents a legal instrument on the subject of accessing to genetic resource and the fair and equitable sharing of benefits arising from their utilization (Myburgh, 2011). DNA barcoding could represent a valid tool to support the adoption of Nagoya protocol, especially in developing countries that are more and more interested in safeguarding their biodiversity and valorize traditions and usages about NUS and other local genetic sources.

On the whole, we can consider DNA barcoding as a valuable tool to valorize NUS for three main aspects.

- Knowledge diffusion and transfer.

Most NUS species are cultivated and used at the local scale, sometimes only by small communities inhabiting a certain geographic region or even by groups of families due to ancient cultural transmission episodes. In other cases, their use is much more common, but they are known under different trivial names, thus impeding a reliable diffusion and conservation effort. A suitable DNA-based identification approach allows to reach standardization and thus to develop a system able to unambiguously identify the genetic resources and assign them to a certain name (Galimberti et al., 2014). Moreover, any deposited reference barcode sequence could be integrated with information on provenance and features such as productivity, water, and soil requirement and the resistance to pests and environmental stress conditions. Such data could improve the worldwide distribution of NUS and a dedicated DNA barcoding database for those species could also have a great impact on the agronomic and scientific community. An established species (and varieties) database is indeed the first step to stimulate research programs on plants genetics and physiology to improve

crop yield in different climatic regions.

- Standardization of identification processes.

DNA barcoding has rapidly affirmed its efficacy among different categories of stakeholders and is nowadays a gold standard for the global food market. In fact, it was proposed by the US Food and Drug Administration for the authentication of food items such as the seafood (Shehata, Naaum, Garduno, & Hanner, 2018). Similarly, DNA barcoding could play a key role in verifying the identity, mislabeling and safety of herbal products used as food or food supplements (Raynor, Dickinson, Knapp, Long, & Nicolson, 2011). We expect that in the next future this approach will be widely applied to several food items, also in response to the adoption of the Nagoya Protocol. In this framework, a universal and standardized system with great economic and political resonance could certainly be adopted to certify NUS and even their varieties.

- Universality of the method.

The universality of the DNA barcoding allows the application of similar protocols for the identification of different matrices, significantly simplifying the analytical approach to food analysis. Furthermore, this method could facilitate the access of smart analytical systems to all the supply chain steps up to the final consumer. For example, many studies coupled the universality of DNA barcodes with the sensitivity of High Resolution Melting (HRM) to authenticate plant products (see for example Madesis, Ganopoulos, Anagnostis, & Tsaftaris, 2012; Sun, Li, Xiong, Zhao, & Chen, 2016). More recently, Valentini et al. (2017) demonstrated that the precision of DNA barcoding can be easily coupled with the high sensitivity of nanotechnologies to provide rapid and user-friendly colorimetric systems to achieve food authentication (Valentini et al., 2017). These kinds of innovations will make molecular-based identification affordable to non-specialized personnel, also in those regions where high-tech laboratory facilities are not available. In particular, in contexts where NUS are getting more diffused, these types of innovation are pivotal to ensure food safety in the supply chain and to avoid adulteration events.

3. Conclusions

It is clear that a sustainable future of agriculture is realizable only by exploring biodiversity at the species and variety levels. Crop genetic diversity is only a key dimension of overall biodiversity. The re-discovery of NUS represents a pivotal element to introduce new plants for cultivation at the global scale. We can consider this strategy a sort of modern green revolution that starts from plant biodiversity to improve farming systems but also considers the cultural, social, political, and economic issues of each interested region. Although these species have seeds, leaves or fruits rich in micronutrients, some of them could resemble other taxa having indigestible parts or secondary metabolites that could be not suitable to human nutrition. Such food safety issues can nowadays take advantage of reliable identification systems like the DNA barcoding one, allowing to assess NUS biodiversity and their distribution. This possibility ensures protection to consumers, producers, and all the stakeholders involved in the NUS supply chain against adulteration and species substitution events. At the same time, the DNA barcoding authentication of NUS contributes to preserve their biodiversity, ethnobotanical knowledge and agricultural sustainability by enhancing farm-level resilience to ongoing global environmental phenomena, such as climate change.

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None.

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